REMARKS

Reconsideration of the claims in view of the following Remarks is respectfully requested. Claim 1 has been amended. Support for the amendment can be found in Figure 5 and Example 3 of the specification, including at page 65, lines 27-29; page 66, line 19 through page 68, line 31. Claims 32-37 are pending in the application.

35 U.S.C. 112, first paragraph

Claims 32-37 were rejected under 35 U.S.C. 112, first paragraph for lack of enablement. The Examiner asserts that prorelaxin is not adequately characterized by structural or chemical means. Applicants traverse this rejection.

There are many factors to be considered in an enablement analysis, including the breadth of the claims, the nature of the invention, the state of the prior art, the level of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and the quantity of experimentation. *MPEP* 2164.01(a) citing *In Re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). The test for undue experimentation "is not merely quantitative, since a considerable amount of experimentation is possible, if it is merely routine, or the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *MPEP 2164.06.* Indeed, "[t]he fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." *MPEP 2164.01.*

The present claims are directed to an isolated animal host cell that is not naturally capable of forming secretory granules, and that comprises a first nucleic acid encoding a prorelaxin polypeptide comprising an amino acid sequence of SEQ ID NO: 59 or SEQ ID NO:60 or mutants thereof having a conservative amino acid substitution at one or more residues, wherein the prorelaxin polypeptide or mutants thereof comprise three polypeptide chains, A, B, and C, and comprise two dibasic enzyme cleavage sites, one positioned at a C-A peptide junction, and one positioned at a C-B peptide junction, and wherein the prorelaxin polypeptide or mutants thereof exhibit a hormonal activity of relaxin; and a second nucleic acid encoding an enzyme that is capable of cleaving the prorelaxin polypeptide to form a mature two chain relaxin polypeptide.

Applicants contend that one of skill in the art reading the specification would be able to practice the claimed invention in light of the enablement standards discussed above, without undue experimentation. Applicants note that the pending claims now refer to prorelaxin polypeptides that: 1) comprise one of two specific sequences, or conservative mutants thereof, 2) that exhibit a hormonal activity of relaxin, and 3) that possess dibasic enzyme cleavage sites positioned at the C-A peptide junction, and another dibasic enzyme cleavage site positioned at the C-B peptide junction.

The Examiner maintains that only rat, shark, porcine, and human prorelaxin had been sequenced at the time of the invention. The Examiner asserts that undue experimentation would be required to determine what residues could be conservatively substituted while preserving biological functionality. The Applicants respectfully disagree with the Examiner's conclusions.

As an initial matter, the Applicants submit with this Response two references demonstrating that equine relaxin (Stewart et al., *Endocrinology*, 1991 Jul;129(1):375-83), as well as the cDNA for rhesus monkey preprorelaxin (Crawford et al., *J. Mol. Endocrinol.*, 1989 Nov;3(3):169-74), had also been sequenced at the time of the present invention. Therefore, no fewer than seven relaxin, prorelaxin, and/or preprorelaxin sequences were publicly available at the time of filing. As a result, the specification, in light of the knowledge of the prorelaxin sequences known in the art, fully enables the claims, and provides guidance concerning residues and domains that may be conservatively substituted while maintaining biological activity.

The Applicants enclose with this Response a sequence alignment showing the sequences of several prorelaxins (human, monkey, pig, and rat) whose sequences were 1) known at the time of the invention, and 2) were known to retain biological activity. The Applicants respectfully submit that the sequence alignment demonstrates that multiple amino acid multiple substitutions, and particularly conservative substitutions as claimed, can be made to prorelaxin without a loss of biological activity.

Therefore, the Applicants respectfully submit that one of ordinary skill in the art could have readily generated a sequence alignment of prorelaxins, relaxins, and/or preprorelaxins whose sequences were known at the time of the invention, and could have readily used the sequence alignment to identify regions of prorelaxin that were conserved among species, as well as the specific locations and identities of amino acid residues that varied among biologically

active relaxins. Consequently, one of skill would have been able to reasonably predict specific conservative substitutions that would not result in the loss of activity, as required by the claims. The Applicants respectfully submit that making these predictions using sequence alignments was routine in the art and would not require undue experimentation, particularly in light of the fact that even extended or complex experimentation may not be undue if it is routine in the art.

Moreover, the specification provides additional guidance in making and using the claimed invention, by providing a working example showing the generation of multiple prorelaxin variants that retained the wild type polypeptide's ability to undergo further processing into a mature form when in the presence of an appropriate cleavage enzyme (see Example 3).

For the foregoing reasons, the Applicants submit the claims are fully enabled in light of the *In Re Wands* factors discussed above. Specifically, the claims refer to specific amino acid sequences, mutants that comprise amino acid substitutions that are conservative in nature, nthe claimed polypeptide exhibits a hormonal activity of relaxin, and the claimed polypeptides comprise structural features in the form of two dibasic cleavage sites at specific junctions. Second, the specification provides actual working examples, including the generation of several biologically active prorelaxin variants that would have provided one of ordinary skill with guidance in selecting variants of the claims that would have retained activity. Third, the Applicants have also shown the existence of knowledge in the prior art regarding multiple prorelaxin sequences known to exhibit biological activity. Fourth, the Applicants have shown that the ability to generate sequence alignments using prorelaxin sequences known in the art provided predictability in the art, such that one of ordinary skill could have predicted conservative amino acid substitutions that could be made to wild-type prorelaxin without eliminating biological activity without undue experimentation.

Although the Applicants believe the foregoing arguments demonstrate that the claims are fully enabled, the Examiner's attention is also respectfully directly to *Capon v. Eshhar*, (418 F.3d 1349 (Fed. Cir. 2005)), for additional evidence in support of Applicants' arguments. Although *Capon* relates to claim rejections that were based upon an alleged lack of written description, the Federal Circuit noted that the Board of Patent Appeals and Interferences had confused issues of written description with issues of enablement in its analysis. Therefore, the Court's analysis

includes statements that are relevant to the present enablement rejection, as will be discussed below.

The fact pattern of *Capon* involved an interference where the designated count was a claim directed to a chimeric gene comprising a first gene segment encoding an scFV of an antibody, and a second gene segment encoding all or part of a domain from an endogenous protein that is expressed on the surface of immune system cells, so as to trigger activation or proliferation of the cells. *Id.* at 1352. The Board declared that the designated count was inadequately described. Specifically, the Board concluded that both specifications failed to sufficiently describe the full scope of the claims, because they did not include the complete nucleotide sequence of at least one chimeric gene encompassed by the claims.

The Federal Circuit, however, vacated and remanded the decision. In concluding that the specification need not specify the sequence of any chimeric gene, the court noted that the invention did "not concern the discovery of gene function or structure," but rather the "the novel combination of the DNA segments to achieve a novel result." *Id.* at 1358. As a result, the court emphasized that the invention was generic in nature, such that it "is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention." *Id.* at 1359.

Specifically, the court asserted that

> "[t]he Board did not discuss the generic concept that both Capon and Eshhar described-the concept of selecting and combining a gene sequence encoding the variable domain of an antibody and a sequence encoding a lymphocyte activation protein, into a single DNA sequence which, upon expression, allows for immune responses that do not occur in nature. The record does not show this concept to be in the prior art, and includes experimental verification as well as potential variability in the concept." *Id.* at 1360.

In response to the Director's assertion that the claims were so broad as to possibly include inoperative embodiments, and that the specifications represented "merely an invitation to experiment," the court noted that the Board's reasoning "concerns enablement more than written description," and that "the Board's repeated observation that the full scope of all the claims appears to be 'enabled' cannot be reconciled with the Board's objection that only a 'general plan' to combine unidentified DNA is presented." *Id.* at 1360. In any case, the court asserted that the Board's reasoning was incorrect, since the disclosures provided a detailed description of

the creation and expression of products from the chimeric genes, including working examples along with "standard systems of description and identification, as well as known procedures for selecting, isolating, and linking known DNA segments." *Id.* at 1360.

The Applicants respectfully submit that *Capon* is relevant to the present case. The Applicants have discovered and disclosed that mature relaxin can be expressed in the host cells recited by the claims, and have provided a detailed working example for the production of relaxin according to the present invention. Applicants' claims are directed to the novel concept that mature relaxin can be produced in the claimed host cells as described in the specification. The Applicants submit, therefore, that the present invention is generalizable in nature, such that prorelaxins other than those specifically disclosed by the specification could also function in the invention as claimed.

For the foregoing reasons, the Applicants respectfully submit that the claims are fully enabled. Withdrawal of the rejection is therefore requested.

35 U.S.C. 103(a)

Claims 32 and 34-37 were rejected under 35 U.S.C. 103(a) as unpatentable over Hudson et al. in view of Mulvihill et al. Applicants traverse this rejection.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: (1) the reference must teach or suggest all of the claim limitations; (2) there must be a suggestion or motivation, either in the reference itself or in the knowledge generally available to one of skill in the art to modify the reference; and (3) there must be a reasonable expectation of success. Applicants submit that not all of these requirements have been met, because there is no suggestion or motivation to modify the references to disclose all of the claim limitations, and because there would be no reasonable expectation of success in doing so.

The present claims recite an isolated animal host cells that is not naturally capable of forming secretory granules, and that comprises a first nucleic acid encoding a prorelaxin polypeptide comprising an amino acid sequence of SEQ ID NO: 59 or SEQ ID NO:60 or mutants thereof having a conservative amino acid substitution at one or more residues, wherein the prorelaxin polypeptide or mutants thereof comprise three polypeptide chains, A, B, and C, and comprise two dibasic enzyme cleavage sites, one positioned at a C-A peptide junction, and one

positioned at a C-B peptide junction, and wherein the prorelaxin polypeptide or mutants thereof exhibit a hormonal activity of relaxin; and a second nucleic acid encoding an enzyme that is capable of cleaving the prorelaxin polypeptide to form a mature two chain relaxin polypeptide. Applicants submit that the cited references, alone or in any combination, do not teach or suggest host cells having all of these limitations.

Hudson et al. describes the cloning and characterization of a gene sequence coding for human prorelaxin and human relaxin. The Hudson et al. reference describes preparation of the A chain and the B chain by synthetic chemical methods. This reference does not teach or suggest that processing of prorelaxin can or should be conducted in a host cell comprising an enzyme that can cleave the prorelaxin into a mature two chain form. This reference nowhere teaches or suggests host cells that comprise a first nucleic acid encoding a prorelaxin polypeptide and a second nucleic acid encoding an enzyme that is capable of cleaving the prorelaxin polypeptide to form a mature two chain relaxin polypeptide.

Mulvihill et al. does not remedy this deficiency. The Mulvihill et al. reference describes the processing of protein C with or without the KEX2 enzyme. As discussed in the previous Response, Protein C differs from prorelaxin not only in its sequence, but also in that it possesses only a single cleavage site having a sequence that differs from that of prorelaxin. Moreover, Protein C is secreted via the constitutive pathway.

By way of contrast, relaxin is synthesized as a preprohormone precursor that is typically processed through the regulated pathway of secretion. This processing does not normally occur when the precursor is heterologously expressed in cells containing only the constitutive pathway of protein secretion (page 7, lines 18 to 26 of the specification). Moreover, processing of prorelaxin requires sequential cleavage at two different cleavage sites (see page 68, lines 21-25; page 69, lines 4-8; and Figure 5 of the specification), including endoproteolytic cleavage at specific pairs of basic amino acid residues, along with the joining of the A and B chains via disulfide bonds with an intra-chain disulfide loop in the A-chain (page 9, lines 7-10). Furthermore, the sequence at each of the cleavage sites differs from that of Protein C.

The Applicants have discovered, however, that prorelaxin can be properly processed to mature relaxin in cells having a constitutive secretion pathway and lacking in the ability to form secretory granules. One of skill in the art would not be motivated to combine or modify Hudson

with the disclosure of Mulvihill, nor have any reasonable expectation of success in doing so, because the protein in Mulvihill is processed differently than that of prorelaxin. As discussed above, Protein C is secreted via the constitutive pathway. Processing of proteins through the constitutive pathway is very different than the processing of proteins through the regulated pathway. There is no teaching or suggestion in Mulvihill that a protein normally processed through the regulated pathway would be correctly folded outside of the secretory granules. Nor is there any teaching or suggestion that a protein normally processed through the regulated pathway would be compartmentalized with the processing enzyme or that the processing enzyme would be concentrated enough to cleave the protein.

In addition, the protein described in Mulvihill has a single cleavage site with a different cleavage sequence than that of prorelaxin. As discussed in Thomas et al, proper processing of proteins that are normally secreted via the regulated pathway may require proper cleavage site structure and accessibility to the cleavage site acting in conjunction with differential expression of a core of processing enzymes. See Thomas at page 5301, end of first paragraph.

There is no teaching or suggestion in Mulvihill that a processing enzyme such as KEX2 could cleave a protein such as prorelaxin when produced in a host lacking secretory granules, in light of the fact that prorelaxin is normally processed through the regulated pathway, has multiple cleavage sites which may not be accessible, and has different sequences at each of the cleavage sites (which may not serve as a substrate for the enzyme).

Applicants additionally submit that neither reference teaches a reasonable expectation of success of obtaining a mature two chain form of relaxin in a host cell lacking secretory granules. Hudson et al. does not describe production of prorelaxin or relaxin in any host cell, but rather describes synthesis of each chain using amino acid synthesis and chemical cross linking as described previously. Mulvihill's disclosure is directed to a protein that differs from the claimed protein in secretory pathway, number of cleavage sites and in the sequence of the cleavage sites. Mulvihill describes cleavage of Protein C at a single site with a cleavage site of RRKR with Kexin. In contrast, prorelaxin has two sites both of which differ in sequence from that of Protein C. The first site at the B/C junction is KR and at the second site RKKR at the C/A junction. Moreover, if either residue at the KR site is an alanine, no processing of prorelaxin occurs at all. Protein C and prorelaxin differ structurally and are processed differently. Protein C and

prorelaxin do not have similar functions. Thus, Applicants submit that even combining Hudson et al. with that of Mulvihull et al. does not disclose all of the elements of the claimed invention.

In response to the foregoing arguments, the Examiner states that the obviousness rejection is maintained, because Protein C possesses a site that is cleavable by the prior art enzyme, regardless of whether the site is identical to that of prorelaxin.

The Applicants respectfully disagree with the Examiner's reasoning, and reiterate the nature of post-translational processing of prorelaxin, as discussed above. One of ordinary skill could not have reasonably predicted that mature relaxin could have been generated in the expression system of Mulvihill merely because Mulvihill discloses that the expression system could produce mature Protein C. At the time of the invention, the precise requirements and mechanisms for proper post-translational processing of protein hormones, such as prorelaxin, were unknown.

It is the Applicants' understanding that the obviousness rejection relies in part on the alleged existence of a structural similarity between prorelaxin and Protein C, i.e., a cleavage site that is cleavable by the same enzyme, even if the cleavage sequence is not identical. As discussed above, Protein C and prorelaxin are not structurally or functionally similar.

In the present case, and as discussed above, prorelaxin and Protein C possess multiple structural differences that preclude any finding of a reasonable expectation of success when the cited references are combined in the manner suggested by the Examiner. Specifically, those structural differences include:

Protein C	**Prorelaxin**
single cleavage site	two cleavage sites
sequence of cleavage site of mutant Protein C RRKR	two sites with sequence at two different sequences B/C chain KR
	sequence at C/A chain RKKR or KKRR
	cleavage is sequential

cleavage does not occur at
RKKR site unless first site is
cleaved KR site

pathway is secretory

Moreover, these two molecules undergo processing differently. Protein C is constitutive and prorelaxin is secretory. There is no teaching or suggestion to use Kexin to cleave proteins that are normally secreted by the secretory pathway. Finally, these two molecules have totally different functions. It would not have been known at the time of the invention whether these differences would affect proper processing of prorelaxin upon expression in a cell naturally lacking secretory granules.

In response to Applicants' previous arguments, the Examiner also contends that whether a peptide's secretion is regulated or constitutive has no apparent bearing on why the present invention is not obvious. The Applicants respectfully disagree with this conclusion as well, and submit with this Response, Colomer et al. (*The EMBO Journal* vol. 13 no. 16 pp. 3711-3719, 1994).

Colomer et al. reveals that both prior to and subsequent to the priority date of the present application, there was no consensus regarding the sorting signals that directed a given secretory protein into either a regulated pathway of secretion or a constitutive pathway. Colomer et al. concludes that their results "strongly support selective co-aggregation as being central in the segregation of constitutive from regulated proteins" (see page 3718, last paragraph). Colomer et al. does not disclose, however, that the co-expression of a heterologous enzyme capable of cleaving prorelaxin into its mature form would allow production of relaxin in cells naturally lacking the ability to form secretory granules, or that a cleavage enzyme played a key role, or any role, in sorting a peptide into a regulated secretory pathway.

Consequently, since 1) prorelaxin is normally not processed correctly by a constitutive secretory pathway, and 2) the mechanism that directs peptides such as prorelaxin to a regulated rather than a constitutive secretory pathway was controversial and unclear, the skilled person could not have reasonably predicted that co-expressing prorelaxin and an endogenous enzyme capable of cleaving prorelaxin, in a host cell not naturally capable of forming secretory granules, would be sufficient by itself to render the cell capable of secreting mature relaxin.

Based on the foregoing, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 103.

Claims 32-37 were rejected under 35 U.S.C. 103(a) as unpatentable over Hudson et al. in view of Mulvihill et al., and further in view of Thomas et al. Applicants traverse this rejection for the same reasons discussed above, and because Thomas et al does not remedy the deficiencies of Mulvihill et al. or Thomas et al.

The teachings of Hudson et al. and Mulvihill et al. have been discussed above. Applicants submit that these references, alone or in combination, fail to provide any motivation to combine the teachings of the reference, nor do they provide any reasonable expectation of success in doing so. Specifically, one of skill in the art could not have reasonably expected that the host cells used in the methods of Mulvihill et al. would be capable of coordinating the cleavage and disulfide-bond forming events necessary to produce mature relaxin from prorelaxin, merely because the methods of Mulvihill et al. were successful in producing a completely different protein secreted via a different pathway and with a completely different cleavage site.

Regarding Thomas et al, this reference fails to remedy the deficiencies of Hudson et al. and Mulvihill et al. Thomas et al. describes the processing of neuroendocrine hormone propiomelanocortin in BSC-40 cells in the presence or absence of different enzymes. There is no teaching or suggestion in this reference that another hormone produced in a different cell type would be processed similarly. As discussed previously, prorelaxin requires a sequential cleavage at multiple cleavage sites, and a disulfide bond that joins the A and B chains. There is no teaching or suggestion that once prorelaxin is produced in a cell without secretory granules, that both of the cleavage sites would be accessible or that the sequence of each cleavage site would serve as a substrate for the processing enzyme.

As mentioned above, Thomas indicates that proper processing includes cleavage site structure and accessibility to the cleavage site acting in conjunction with the processing enzyme. Moreover, the Thomas et al reference shows that the different prohormone convertase enzymes were not able to cleave at every site and completely process the prohormone. Therefore, the Applicants respectfully submit that Thomas et al. demonstrates the lack of any reasonable

expectation of success in substituting prorelaxin for Protein C in the methods disclosed by Mulvihill et al.

Applicants submit that claims 32-37 are patentable over the cited references, at least for the foregoing reasons. Withdrawal of the rejection is requested.

INTERVIEW

Applicants request an interview with the Examiner and his supervisor.

SUMMARY

Applicants submit that the claims are in condition for allowance and notification to that effect is earnestly solicited. The Examiner is invited to contact Applicants' representative if prosecution may be assisted thereby.

Respectfully submitted,

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